Supporting Information

Mild and Efficient Strategy for Site-selective Aldehyde Modification of Glycosaminoglycans: Tailoring Hydrogels with Tunable Release of Growth Factor

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1. Materials and methods:

Hyaluronic acid (HA, 150 kDa) was purchased from Lifecore Biomedical, LLC (Chaska, MN). Heparin (HP, 15 kDa from porcine intestinal mucosa) and chondroitin sulphate-A (CS-A, 54 kDa from bovine trachea) were purchased from Sigma-Aldrich (Sweden). N-hydroxybenzotriazole (HOBt), sodium perioxide ($NaIO_4$), carbodihydrazide (CDH), bovine serum albumin (BSA), hyaluronidase, ethylene glycol, 1-ethyl-3-(3-dimenthylaminopropyl) carbodiimide (EDC) were purchased from Sigma-Aldrich (Sweden). Human basic fibroblast growth factor (FGF-2) and ELISA kit of FGF-2 were from Invitrogen, dialysis membrane Spectra/Pro 3 (3500 g/mol cut off) and Spectra/Pro 7 (1000 g/mol cut off) were purchased from Spectrum Laboratory Inc. All the materials used in this article were analytic reagent (AR) grade and used as received.

The NMR experiments (δ scale; J values are in Hz) were carried out on Jeol JNM-ECP Series FT NMR system at a magnetic field strength of 9.4 T, operating at 400 MHz for ^1H. Rheology measurements were carried out on AR2000 Advanced Rheometer (TA Instruments). Spectroscopic analyses were carried out on Spectrum One AT-FTIR and Lambda 35 UV-Vis spectrophotometer.

2. Periodate oxidation kinetics

Periodate oxidation kinetics of native and amino glycerol modified glycosaminoglycans (GAGs) were investigated by measuring the consumption of periodate over time.[1] Concentration of unreacted periodate was determined from the standard curve with different known concentrations (0.2 to 7 mM) of periodate (Figure S1).

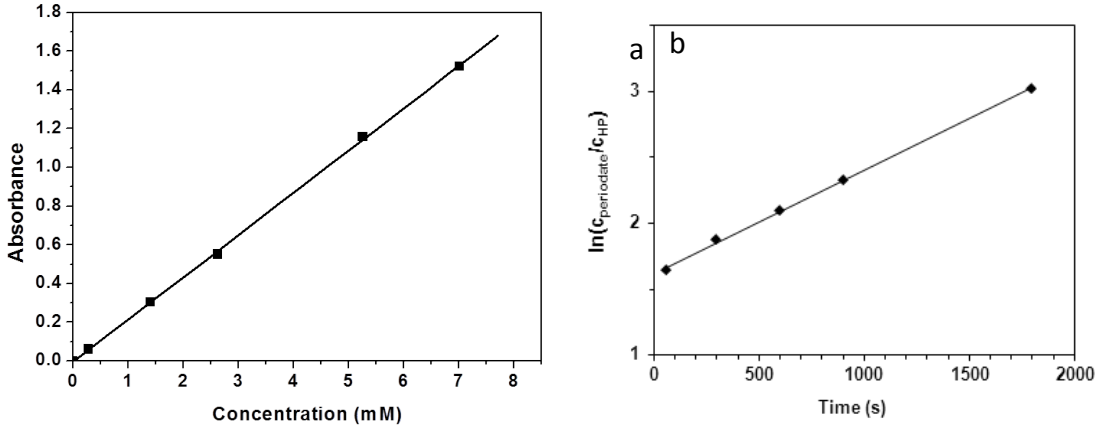


Figure S1. (a) Standard curve of periodate absorbance at 290 nm; (b) Kinetic plot for HP-aminoglycerol oxidation having 20% modifications.

3. Synthesis of hydrazide modified HA

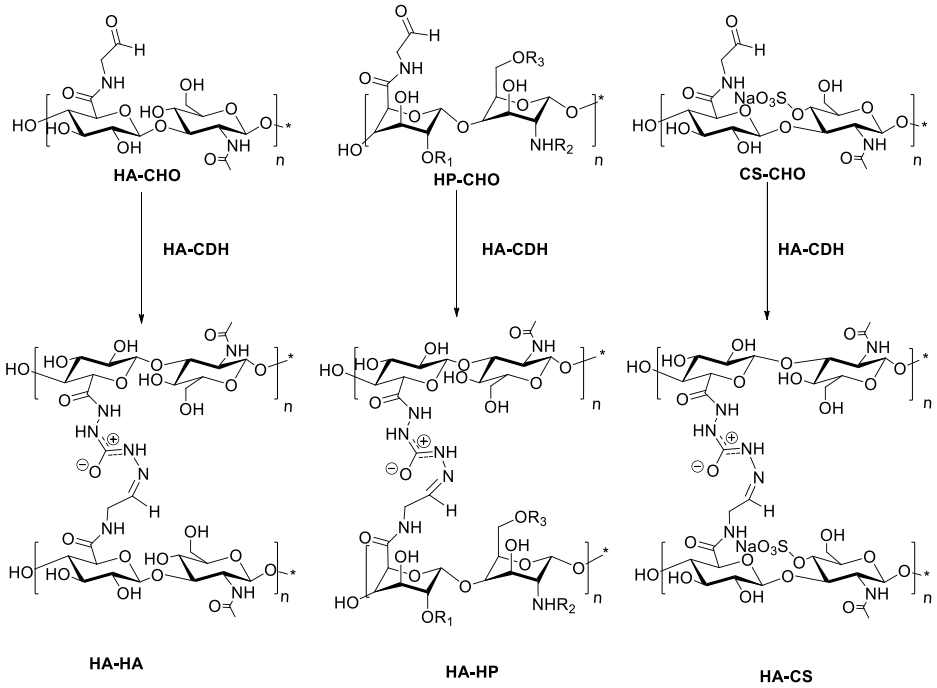
Synthesis and characterization of HA-hydrazide using carbodihydrazide (CDH) has been reported in our recent publication.[2]

4. Preparation of hydrogel

Hydrazone crosslinked HA gel showed good mechanical properties and stability in previous experiment. In this project GAG-aldehyde derivatives (~10% modification) and HA-CDH derivatives (13.2% modification) were used to prepare hydrazone cross-linked hydrogel.

HA-aldehyde (HA-CHO), HP-aldehyde (HP-CHO) and CS-aldehyde (CS-CHO) and HA-hydrazide (HA-CDH) were separately dissolved in PBS (pH 7.4) to reach a concentration of 16 mg/mL. HA-HA gel was prepared by mixing same volume of HA-CHO and HA-CDH

solution by votexing. In HA-HP gel and HA-CS gel, 50 wt% of HA-CHO solution was replaced by HP-CHO or CS-CHO (Table S1 and Scheme S1). The solution was inject to cylinder mould immediately after mixing and covered carefully with Parafilm M. All gels were incubated 24 hours at room temperature before use.



Scheme S1. Hydrazone crosslink between GAG-CHO and HA-CDH

Table S1. Sample Index and Composition of Hydrogels

Sample	Composition (w/w)
HA-HA	HA-CHO:HA-CDH =1:1
HA-HP	HA-CHO:HP-CHO:HA-CDH =1:1:2
HA-CS	HA-CHO:CS-CHO:HA-CDH =1:1:2

5. IR Analysis

Hydrazone crosslinked GAG hydrogels were prepared as described above, frozen in liquid nitrogen and lyophilized. These samples were used to measure attenuated FTIR spectra.

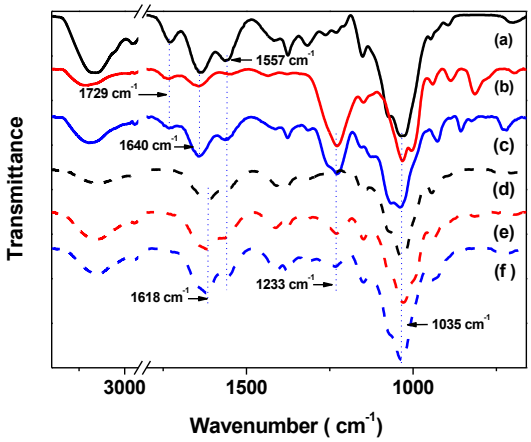


Figure S2. ATR-FTIR spectrum of a) HA-CHO, b) HP-CHO, c) CS-CHO, d) HA-HA gel, e) HA-HP gel and f) HA-CS gel.

6. Swelling and degradation studies

To determine hydrolysis stability and swelling property of gels, 300 µl scale fully cured hydrogels were prepared as described. Gels were weighed and dispersed in 4 ml of PBS (pH 7.4). At different time points, gels were weighed after remove medium. All the experiments were performed in triplicate. Percentage swelling ratio (sw%) was expressed as the percentage ratio between the weight of swollen gel and fresh gel. It could be derived from Eq S1.

$$sw\% = \frac{w_t - w_0}{w_0} \times 100\% \quad sw\% = \frac{w_t - w_0}{w_0} \times 100\% \quad \text{(S1)}$$

wt is the weight of gel after swelling time t, w0 is the weight of gel directly after hydrogel preparation. Each experiment was performed in triplicate.

Enzymatic degradation test was performed in PBS buffer pH 7.4 in the presence of hyaluronidase (25 U/ml). Briefly, 300 µl scale fully cured gels were incubated in 2mL PBS buffer containing hyaluronidase at room temperature. The incubation medium was changed daily and the time when the gel disappeared was recorded. All the experiments were performed in triplicate.

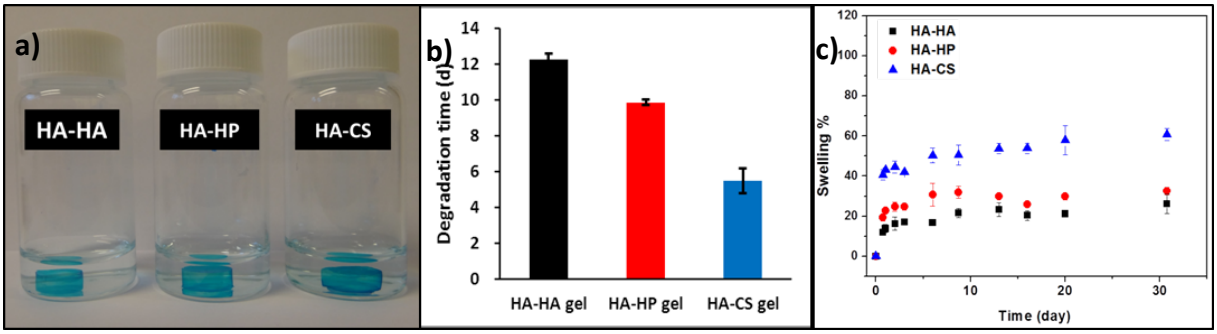


Figure S3 a) Hydrogels stained with alcain blue after swelling in PBS for 30 days; b) Hyaluronidase mediated enzymatic degradation of hydrogels with time; c) Swelling ratio of hydrogels as a function of time.

7. Rheological measurements

Hydrogels were prepared as describe. The dynamic viscoelastic behaviours of hydrogel were investigated by using AR2000 Advanced Rheometer (TA Instruments) and 8 mm diameter parallel plate geometry. Oscillatory frequency sweep tests were carried out at constant oscillatory stain (1%), normal force (20mN) at room temperature with frequency varied between 0.01 and 10 Hz to maintain the measurements within the linear viscoelastic region (LVER). Rheology tests were performed on fresh hydrogel and gels after swelling in PBS (pH 7.4) for 24 hours. All the experiments were performed in triplicate.

We extracted the average mesh size (ξ) as well as the average molecular weight between crosslinks (Mc) in the hydrogel from peak value of storage modulus in viscoelastic region.3,4 The average mesh size (distance between two crosslinks or entanglement points) ξ was calculated based on rubber elastic theory that can be applied on hydrogels that has elastic character.5 The Mc and ξ parameters were derived from the following eq S2 and eq S3:

$$Mc = \frac{c\rho RT}{G'_p} \quad \text{(S2)}$$

$$\xi = \left(\frac{G'N_A}{RT}\right)^{-1/3} \quad \text{(S3)}$$

where c is the polymer concentration (1.6 % w/v), ρ is the density of water at 298 K (997 kg.m-3), R is molar gas constant, G'_p is the peak value of G' in linear viscoelastic region, NA is the Avogadro constant and T is temperature (298 K).

References:

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